Items needed for protocol (bold items are from Zymo Research):

- Reagent needed
  - **DNA/RNA Shield** (R1100-50)
  - **MagBead DNA/RNA Wash 1** (R2130-1-30) with isopropanol added
  - **MagBead DNA/RNA Wash 2** (R2130-2-20) with isopropanol added
  - **Viral DNA/RNA Buffer** (D7020-1-50)
  - **Proteinase K with storage buffer** (D3001-2-5)
  - **DNase/RNase Free Water** (W1001-30)
  - **MagBinding Beads** (D4100-2-3)
  - 95-100% Ethanol
- Tips
  - 1,000 µL filter tips
  - 50 µL filter tips
- Hardware / Labware
  - **96-Well Block** (P1001-2)
    - 2 mL capacity/well
  - **Collection Plates** (C2002)
    - 1.2 mL capacity/well
  - **Elution Plates** (C2003)
    - Polypropylene skirted plate with "V" bottom
  - Waste Trough (Example: Agilent Part# 201244-100)
    - Optional SBS trough to dispense liquid waste
  - On-Deck Heater/Shaker (optional)
  - Magnetic Stand (Recommended: Alpaqua FLX Magnum)
- Instrument pipetting capability
  - If 96-head does not have 1,000 µL pipetting capability, all the wash buffer and ethanol wash volumes can be cut in half. See APPENDIX Step 9 for recommendation about binding step mixing.

Reagent and Sample Preparation:

See page 3 of kit protocol for details on reagent preparation and sample preparation.

Scripting Logic:

DNA/RNA Purification

1. Sample Plate: 200 µL of cleared sample per well of the **96-Well Block**
   Note: Sample input was lowered to accommodate a 1 mL processing volume limitations that many automated platforms require.
2. Pick up 50 µL tips
3. Aspirate and dispense 2 µL **Proteinase K** to samples in each well of the **96-Well Block**
   Note: due to low volume, contact dispense may be needed
4. Mix well by pipetting and/or shaking with On-Deck Heater/Shaker
   Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
5. Discard tips
6. Pick up 1,000 µL tips
7. Aspirate and dispense 400 µL of **Viral DNA/RNA Buffer** to samples in the **96-Well Block**
8. Mix well by pipetting and/or shaking with On-Deck Heater/Shaker
   Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
9. Discard tips
10. Pick up 50 µL tips
11. Premix **MagBinding Beads** by pipette mixing at very fast speed for 20 aspirate/dispense cycles
12. Aspirate and multi-dispense 20 µL of **MagBinding Beads** to each well of the **96-Well Block**
13. Discard tips
14. Mix well for 10 minutes by shaking with on-deck heater/shaker

Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
Alternatively, a new set of 1,000 µL tips can be used to mix the MagBinding Beads and sample.

15. Transfer **96-Well Block** to magnetic stand
16. Incubate for 2-5 minutes until supernatant is cleared.
    Note: Time will vary depending on Magnetic Stand and may need optimization.
17. Pick up 1,000 µL tips
18. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
19. Discard supernatant in Waste Trough.
    Note: Pipetted tips should dispense below the top of the Waste Trough to prevent splattering, which may contaminate adjacent labware.
20. Discard tips
21. Remove **96-Well Block** from magnetic stand and move to empty carrier
22. Pick up 1,000 µL tips
23. Aspirate and dispense 500 µL of **MagBead DNA/RNA Wash 1** to each well
24. Mix well by pipetting and/or shaking with on-deck heater/shaker for 2 - 4 minutes
    Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
25. Discard tips
26. Transfer **96-Well Block** to magnetic stand
27. Incubate for 2-5 minutes until supernatant is cleared.
    Note: Time will vary depending on Magnetic Stand and may need optimization.
28. Pick up 1,000 µL tips
29. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
30. Discard supernatant in Waste Trough.
    Note: Pipetted tips should dispense below the top of the Waste Trough to prevent splattering, which may contaminate adjacent labware.
31. Discard tips
32. Remove **96-Well Block** from magnetic stand and move to empty carrier
33. Pick up 1,000 µL tips
34. Aspirate and dispense 500 µL of **MagBead DNA/RNA Wash 2** to each well
35. Mix well by pipetting and/or shaking with on-deck heater/shaker for 2 - 4 minutes
    Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
36. Discard tips
37. Transfer **96-Well Block** to magnetic stand
38. Incubate for 2-5 minutes until supernatant is cleared.
    Note: Time will vary depending on Magnetic Stand and may need optimization.
39. Pick up 1,000 µL tips
40. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
41. Discard supernatant in Waste Trough.
    Note: Pipetted tips should dispense below the top of the Waste Trough to prevent splattering, which may contaminate adjacent labware.
42. Discard tips
43. Remove **96-Well Block** from magnetic stand and move to empty carrier
44. Pick up 1,000 µL tips
45. Aspirate and dispense 500 µL of 95-100% ethanol to each well
46. Mix well by pipetting and/or shaking with on-deck heater/shaker for 2 - 4 minutes
    Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
47. Discard tips
48. Transfer **96-Well Block** to magnetic stand
49. Incubate for 2-5 minutes until supernatant is cleared.

Note: Time will vary depending on Magnetic Stand and may need optimization.

50. Pick up 1,000 µL tips
51. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
52. Discard supernatant in Waste Trough.
    Note: Pipetted tips should dispense below the top of the Waste Trough to prevent splattering, which may contaminate adjacent labware.
53. Discard tips
54. Remove **96-Well Block** from magnetic stand and move to empty carrier
55. Pick up 1,000 µL tips
56. Aspirate and dispense second wash of 500 µL of 95-100% ethanol to each well
57. Mix well by pipetting and/or shaking with on-deck heater/shaker
    Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
58. Discard tips
59. Transfer **96-Well Block** to magnetic stand
60. Incubate for 2-5 minutes until supernatant is cleared.
    Note: Time will vary depending on Magnetic Stand and may need optimization.
61. Pick up 1,000 µL tips
62. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
63. Discard supernatant in Waste Trough.
    Note: Pipetted tips should dispense below the top of the Waste Trough to prevent splattering, which may contaminate adjacent labware.
64. Discard tips
65. Dry beads on magnetic stand by incubating at room temperature for 10 minutes or until beads are fully dry
    Note: Beads will change in appearance from glossy black when still wet to a matte/dull brown when dry. If beads turn a rusty/flaky appearance, they are over-dried. Dry time will vary depending on room temperature/humidity that the instrument is in and should be empirically tested during initial setup.
66. Remove **96-Well Block** from magnetic stand and move to empty carrier
67. Pick up 50 µL tips
68. Aspirate and dispense 50 µL of **DNase/RNase Free Water** near the bottom of **96-Well Block** wells.
    Note: 30 µL of DNase/RNase Free Water may be used for highly concentrated DNA/RNA. This will require optimization of liquid classes, pipetting speeds, position of pipette tips, and the magnet stand that is being used. This should be empirically tested during initial setup.
69. Mix well for 5 minutes by pipetting and/or shaking with On-Deck Heater/Shaker to completely resuspend the beads.
    Note: Heater-shaker settings will vary. Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity. We recommend a speed between 1200 – 1500 RPM.
70. Discard tips
71. Transfer **96-Well Block** to magnetic stand
72. Incubate for 2-5 minutes until supernatant is cleared.
    Note: Time will vary depending on Magnetic Stand and may need optimization.
73. Pick up 50 µL tips
74. Aspirate supernatant using liquid level detection (if available).
    Note: Use a very slow aspiration speed to avoid aspirating MagBeads.
75. Dispense supernatant in clean **Elution Plate**.
    Note: Pipetted tips should dispense below the top of the plate to prevent contaminating adjacent wells.
76. Discard tips.
77. The DNA and RNA in the **Elution Plate** is now ready for immediate analysis or can be store at ≤ 70°C.

APPENDIX: Troubleshooting and Liquid Class Recommendations:

1. **Pipette mixing liquid class:** Vigorous mixing. Fast aspiration and dispense with no pause in between.
2. **Supernatant removal liquid class:** Slow aspiration speed (10 µL/s) when removing supernatant to avoid aspirating MagBeads.
3. **Elution transfer liquid class:** Aspirate very slowly (5 µL/s).
4. **Bead Resuspension:** Magnetic beads must be completely resuspended into the solution during binding and washing steps for optimal yields and purity.
5. **Foaming prevention:** To prevent foaming of certain buffers, minimize the use of trailing and leading airgaps.
6. **Liquid level detection:** Recommended for supernatant removal if available.
7. **Low yield:**
   - Increasing binding mixing time to 10-20 minutes may improve yields.
   - Shortening drying times may improve yields.
   - If using On-Deck Heater/Shaker, verify that temperatures are correct. Alternatively, dry at room temperature.
8. **Instrument doesn't have 1 mL pipetting capability with 96-head:**
   - If robot only has low volume channels (i.e., ~200 µL max), you can halve all the volumes of Wash Buffers in the protocol. However, the binding step should be full volume.
   - To mix binding step with no shaker, mix the full volume by setting 3 fixed heights: (1) top of well (2) bottom of well (3) middle of well. Make sure the fixed heights at bottom and middle of the well don't cause liquid to spill over out of wells. Pipette mix at each of these 3 heights for 5-10 cycles until beads have been completely resuspended.